bonded via a bridging member,

-Met-Ile-Glu-Gly-Arg-,

to a peptide which stabilizes the fusion protein;

(b) liberating a mini-proinsulin compound from said fusion protein by cleaving the expressed fusion resulting from step (a) with cyanogen bromide to produce mini-proinsulin;

(c) incubating the product formed in step (b) with sodium tetrathionate to form hexa-5-sulfonate;

(d) simultaneously incubating the S-sulfonate mini-proinsulin formed in step (c) with trypsin and carboxypeptidase at a pH of about 6.8 under conditions where no crystals are formed; and

(e) precipitating the insulin,

wherein all of (a) through (e) are performed in one vessel.--

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REMARKS

I. Status of the Claims

Claims 21-23, 25-27 and 31 are currently pending in this application. Applicants amend Claim 31 to more particularly point out and distinctly claim that which Applicants regard as their invention. Applicants add Claim 32 as supported by the specification as a whole and, for example, at page 2, lines 7-20. No new matter enters by these amendments.

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II. Rejections Under 35 U.S.C. § 112, First Paragraph

A. Written Description - New Matter

The Examiner rejects Claims 21-23, 25-27 and 31 under 35 U.S.C. § 112, first paragraph, alleging that the claim limitation "under conditions where no crystals are formed" is new matter, because there is allegedly nothing in the specification which serves as a basis to exclude conditions where crystals are formed. [Office Action at page 3, lines 1-7.] Although the Examiner concedes that this limitation is a property of the examples at pages 15-16 of the specification, the Examiner asserts that this is a negative limitation, which is not implicit in the specification as filed.

It is a well established tenet of patent law that the specification need not describe the claimed invention using the identical words found in the claims in order to satisfy the requirements of 35 U.S.C. § 112, first paragraph. Martin v. Johnson, 172 U.S.P.Q. 391, 395 (C.C.P.A. 1972). Therefore, Applicants respectfully assert that the Examiner is not applying the proper standards set forth in the case law.

First, the C.C.P.A. has addressed the issue of new matter and ruled that "the issue is not whether a specific new word of a claim was used in the specification as filed, but whether the concept expressed by the word was present." In re Anderson, 176 U.S.P.Q. 331 (C.C.P.A. 1973). Accordingly, the issue is not whether the phrase "under conditions where no crystals are formed" has been used in the specification, but whether the concept expressed by this phrase is present in the specification as filed. The Examiner's admission that this limitation is a property of the examples at pages 15-16 is evidence that the skilled artisan would recognize that no crystals are formed

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under the conditions described in the specification. Thus, the concept expressed by the disputed phrase is present in the specification as filed.

In addition, the fact that mono-Arg-insulin is obtained as a precipitate and not in the form of crystals is also confirmed by the crystallization step described in the specification, which is performed after the precipitate is obtained. [Specification at page 16, lines 3-6.] Furthermore, in Tektronix, Inc. v. United States, 165 U.S.P.Q. 392, 394 (Court of Claims 1970), the court ruled that it is not new matter to amend the specification and drawings to make explicit a disclosure which was implicit in the application as filed. Applicants also refer the Examiner to MPEP § 2163.07(a), which states that:

> By disclosing in a patent application a device that inherently performs a function or has a property, operates according to a theory or has an advantage, a patent application necessarily discloses that function, theory or advantage, even though it says nothing explicit concerning it. The application may later be amended to recite the function, theory or advantage without introducing prohibited new matter. *In re Reynolds*, 443 F.2d 384, 170 USPQ 94 (CCPA 1971), *In re Smythe*, 480 F.2d 1376, 178 USPQ 279 (CCPA 1973).

Second, the purpose of the written description requirement is to ensure that Applicants are in possession of the claimed invention as of the date of filing of the application. Vas-Cath Inc. v. Mahurkar, 19 USPQ2d 1111, 1117 (Fed. Cir. 1991). Here, the teachings of the specification described above clearly convey to those skilled in the art that the claimed method of preparing mono-Arg-insulin was indeed in the possession of the Applicants as of the filing date of this application.

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Third, Applicants respectfully submit that MPEP 2173.05(I) is not pertinent. As noted above, the description of process parameters that result in no crystals being formed is the basis for the limitation "where no crystals are formed." The description of such process parameters is not "[t]he mere absence of a positive recitation...." On the contrary, it is a specific description of a process where no crystals are formed.

For these reasons, Applicants respectfully traverse this written description rejection under 35 U.S.C. § 112, first paragraph, and request withdrawal of it.

III. Rejection Under 35 U.S.C. § 103

The Examiner maintains the rejection of Claims 21-23, 25-27, and 31 under 35 U.S.C. § 103 as allegedly being obvious over Markussen *et al.* (U.S. Patent No. 4,916,212 and EP 0153,529) ("Markussen references") in view of Goeddel *et al.*, Mai *et al.*, and Grau (U.S. Patent Nos. 4,801,684 and 4,639,332), for the reasons of record. [Office Action at page 4]. The Examiner argues that Markussen discloses a method of producing insulin as well as insulin precursors. The Examiner asserts that the claimed process is rendered obvious because Grau teaches that mono-Arg-insulin is exceptionally stable.

Applicants respectfully submit that the Examiner has failed to establish a *prima facie* case of obviousness because the applied art would have failed to:

(a) teach or suggest the starting material used in the claimed process;

(b) teach or suggest the formation of mono-Arg-insulin as an intermediate;

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(c) teach or suggest the simultaneous addition of trypsin and carboxypeptidase as presently claimed; and

(d) teach a process of producing mono-Arg-insulin under conditions where no crystals are formed.

In addition, none of the references cited by the Examiner would have taught or suggested a process of preparing insulin without the formation of substantial amounts of insulin Des-B30 as claimed in Claim 31. Applicants continue to rely on arguments already of record supporting these positions. [1]

Applicants now address specific points in the Office Action. In response to Applicants' previous arguments that the existence of mono-Arg-insulin would not have provided the requisite motivation to modify the process disclosed by Markussen, the Examiner contends that since Grau teaches the stability of mono-Arg-insulin to tryptic degradation, the process of Markussen using mono-Arg-insulin would have been obvious to the skilled artisan. [Office Action at page 5.] The Examiner contends that one of ordinary skill in the art would have been motivated to select the species of Grau, which allegedly fits into the starting material genus of Markussen, and to carry out the presently claimed method. The Examiner, however, fails to explain how the prior art of record would have taught combining such a species as the starting material in the Markussen process, and modifying that process to arrive at the claimed process.

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[1] The Examiner did not address this specific limitation in claim 31. If the Examiner maintains the rejection of claim 31, applicants respectfully request the Examiner to address on the record this limitation.

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Applicants submit that the prior art of record would not have suggested using the species of Grau in the Markussen process as alleged by the Examiner. In fact, Applicants wish to clarify the record concerning apparent misperceptions of the Examiner concerning miniproinsulin and mono-Arg-insulin. In the present claims, miniproinsulin is the first starting material and mono-Arg-insulin is the final product in some claims (Claims 21 and 25) or an intermediate in other claims (Claims 22-23, 26-27, and 31).

The Examiner asserts that mono-Arg-insulin is a species of miniproinsulin and is encompassed within the broad genus disclosed by Markussen. Mono-Arg-insulin is not a species of miniproinsulin as alleged by the Examiner. Mini-proinsulin comprises a single amino acid chain comprising the B-, C and A-chain of insulin. Mono-Arg-insulin comprises two amino acid chains connected via disulfide linkages. Moreover, it does not appear that the mono-Arg-insulin of Grau fits within the generic formula of the starting precursor of Markussen (see, e.g., the formula at the bottom of column 2 in the '212 patent). Also, Markussen does not appear to disclose a generic formula that would encompass mono-Arg-insulin at any stage of the process.

Markussen '212 and Markussen (EPO) discuss methods for producing "insulin precursors." (Markussen '212, at col. 2, lines 33-39, for example.) The "insulin precursors" of Markussen differ from the recited mono-Arg-insulin and insulin in that they have not been properly converted into the two chain form of insulin. For example, the "natural" single chain precursor to insulin is the single chain B-C-A polypeptide, where the C chain is removed by proteolytic cleavage to convert the polypeptide into a

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two chain insulin molecule. (See Figure 29-10 in the excerpt of Zubay's "Biochemistry" submitted with the Amendment filed Aug. 2, 1996.) If the Examiner needs another copy of this article, please contact the undersigned.

The required "in vitro conversion" to arrive at a process producing mature human insulin, as discussed in the Markussen documents, refers only to a process involving L-threonine esters. (Markussen '212, at col. 5, lines 3-11; and Examples 14-18 at col. 18, line 25, through col. 19, line 33.) Markussen's use of this method supports a conclusion that those skilled in the art would not have expected trypsin to cleave at the C-terminus of a bridging Arg residue in the single chain "precursor" (the Arg of the recited formula B(1-30)-Arg-A(1-21)) to ultimately generate the two chain, mature form of insulin. Why else would one go through the additional steps involved in the L-threonine ester and not discuss or even mention other more direct methods such as cleavage with trypsin? Accordingly, applicants' invention of methods wherein trypsin can be used in cleaving the single chain "precursor" into the final insulin or mono-Arg-insulin is not taught or suggested by the Markussen documents. In fact the Markussen documents teach different methods that suggest against cleavage with tryspin.

Furthermore, applicants have argued that Thim et al. indicate that trypsin cannot cleave a miniproinsulin with a single Arg bridge between the B and A chains. (Amendment filed November 6, 1995, at page 11.) The Office's response at pages 5-6 of Paper No. 32 appears to confuse the cleavage of "additional protein" in the optional fusion protein construct discussed in Markussen '212 with the enzymatic cleavage that produces insulin from a single chain precursor. In other words, Markussen '212

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discusses such cleavage of a fusion protein at the N terminus of the B chain rather than cleavage of the single chain B(1-29)-X_n-Y-A(1-21) "insulin precursor" to generate the mature, two chain insulin. (See Markussen '212, at col 3, lines 36-41.) For example, "[t]he insulin precursors may be expressed with additional protein proceeding the insulin precursor." (Markussen '212, at col. 3, lines 52-53; see also col. 4, lines 19-24 discussing N-terminal "superfluous amino acid sequence.") The discussion the Office cites from Markussen '212 (col. 4, lines 26-29) relates to that cleavage of an insulin precursor from "an amino acid sequence linked to the B(1-29)-chain" or "additional N-terminal amino acid-sequence to be removed." (Paper No. 32 at page 5.) It does not, however, relate to the enzymatic cleavage step involving the generation of insulin from a single chain precursor. Thus, the Office's comments do not rebut applicants' showing that Thim et al. teach away from the combination the Office proposes since one skilled in the art would not have expected trypsin to cleave at a single Arg residue in the applicants' recited formula B(1-30)-Arg-A(1-21). This enzymatic cleavage results in a two chain insulin or mono-Arg-insulin product according to the claimed invention.

Next, in addition to failing to establish that the art of record would have suggested modifying the methods of Markussen to arrive at the claimed methods for the reasons discussed above, the Examiner has also failed to suggest using the specifically claimed miniproinsulin (B(1-30)-Arg-A(1-21)) in any method, let alone applicants' claimed method. Markussen discusses a precursor represented by a generic formula B(1-29)-$(X_n$-$Y)_m$-A(1-21), which includes a very large number of

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species. In fact, a simple calculation shows this formula includes about 3.6×10^{43}, possible compounds, which may be used as the starting material to produce insulin.

The Federal Circuit has addressed this issue in In re Baird, 15 F.3d 380 (Fed. Cir. 1994). In Baird, the court stated that a disclosure of millions of compounds does not render obvious a claim to three compounds. *Id.* at 1552. The Court held that there was nothing in the prior art suggesting that one of ordinary skill should select the claimed compound from the numerous compounds contained within the broad genus. Applicants respectfully refer the Examiner to the Patent and Trademark Office's new "Genus-Species Guidelines" (62 FR 6217) that became effective on February 11, 1997 changing the Office policy to incorporate the holding in Baird. Thus, based on Baird, the Examiner has provided no reasons why one would have selected the miniproinsulin as the starting material to produce insulin, especially given the large number of possible compounds included within Markussen's generic formula. Stability of mono-Arg-insulin does not render applicants' miniproinsulin obvious, since mono-Arg-insulin does not fit within the generic formula of Markussen as suggested by the Examiner.

The Examiner contends that Grau ('684) teaches the simultaneous addition of trypsin and carboxypeptidase B at Col. 5, lines 57-59. [Office Action at page 7, lines 4-9.] Applicants previously pointed out that Grau ('684) dealt with processes for obtaining insulin precursors rather than processes for obtaining insulin from mono-Arg-insulin. The Examiner disputes this assertion, contending that column 5, line 57-59, discusses using the two enzymes simultaneously to produce insulin. [Office Action at page 7, first full paragraph].

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Applicants point out that the method disclosed in Grau ('684) is different from the method of the present invention since Grau ('684) describes a **natural porcin proinsulin** isolated from pancreas while the instant invention describes a mini-proinsulin of the formula B(1-30)-Arg-A(1-21).

Since it is known that trypsin cleaves at the carboxy terminus of the basic amino acids arginine and lysine, in the case of porcine proinsulin, trypsin can cleave theoretically at 6 different sites in the molecule as indicated in the enclosed figure (2 sites in the B-chain, 3 sites in the C-chain and 1 site between C- and A- chain). Applicants inform the undersigned that, because the cutting rates (kinetics) of the possible cuts depend on the amino acid environment at each site, the actual cutting rates at the six different sites are different. For example, applicants assert that the cutting rates at Arg-(B22) and Lys-(B29) must be relatively low compared to at least the rate of the cutting site at Arg-(C35) since the process described by Grau ('684) yields as main product insulin with intact B-chain. (The Arg-(C1) is cut off by carboxypeptidase B.)

The mini-proinsulin of the process of the present invention is part of a fusion protein. The fusion part is connected via a bridging member (MIEGR) to the mini-proinsulin having a C-chain with just one amino acid (Arg). Cutting off the rest of the bridging member (IEGR) - after removal of the fusion part by CNBr - is provided by trypsin, which cuts also at other sites in the mini-proinsulin part of the fusion protein (Arg-(B22), Lys-(B29) and Arg-(C1)). However, cutting off the mini-proinsulin from the bridging member occurs at a site which has no equivalent in the porcine insulin

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described by Grau ('684). In addition, applicants assert that the cutting rate of trypsin at this new site could not have been predicted in view of Grau ('684). Therefore, Applicants assert that the present invention would not have been obvious, since it was surprising that the relative cutting rate of trypsin at the Arg of the bridging member is high enough compared to the cutting rates at Arg-(B22) and Lys-(B29) to give a good yield of human insulin. If the cutting rates were for example, roughly identical, one could not expect a good yield of intact human insulin, let alone a situation in which the cutting rates at Arg-(B22) and Lys-(B29) were higher than the cutting rate at the Arg of the bridging member.

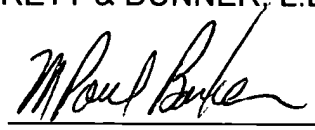
For all of these reasons, applicants respectfully traverse this § 103 rejection and request reconsideration and withdrawal of it.

If there are any fees required in connection with the filing of this response the Commissioner is hereby authorized to charge any additional fees (or credit any overpayment) associated with this response to our Deposit Account No. 06-0916. If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such extension is requested and should also be charged to our Deposit Account.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

Dated: June 6, 1997

By: 

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